

**REMARKS**

Claims 48-49 and 83-124 constitute the pending claims in the present application. Claims 48-49 have been withdrawn, claims 83-87 and 111-112 have been amended, and claims 109-110 have been canceled without prejudice. The claim amendments and additions are fully supported by the specification. No new matter has been introduced. In particular, support for the amendment to claim 1 may be found, for example, at page 3, lines 5-6, etc.

Amendment of claims should in no way be construed as an acquiescence to any of the Examiner's rejections. The amendments to the claims are being made solely to expedite prosecution of the present application and do not, and are not intended to, narrow the claims in anyway. Applicants reserve the option to further prosecute the same or similar claims in the instant or in a subsequent patent application.

Applicants respectfully request reconsideration in view of the following remarks. Issues raised by the Examiner will be addressed below in the order they appear in the prior Office Action.

**Priority**

The Examiner asserts that priority has not been granted for Provisional Application No. 60/189,739 under 35 U.S.C. 119(e) because the provisional application does not provide support for claims 83-124. Applicants reserve the right to claim the priority under 35 U.S.C. 119(e) upon indication of allowable subject matter in the future.

**Double Patenting**

Claims 95-98 and 101 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as allegedly being not patentably distinct over claims 25-28 of U.S. Patent Application No. 10/350,798. Applicants request that the Examiner hold the provisional rejections made under the judicially created doctrine of obviousness-type double patenting in abeyance until otherwise allowable subject matter is identified in the instant application. Once allowable subject matter has been identified,

Applicants will evaluate the filing of a terminal disclaimer or providing arguments in view of the claims pending at that time.

Claim Rejections under 35 U.S.C. § 102:

Claims 183-124 are rejected under 35 U.S.C. § 102(e) as allegedly being anticipated by Fire et al. (U.S. Patent No. 6,506,559). Applicants respectfully traverse the rejection.

At the time of the priority date of the present application, RNA interference (RNAi) had been demonstrated to be extremely active in several invertebrate species. However, while it may have been highly tempting to attempt to adapt this technology to mammalian cells, it had been demonstrated at that time that mammals, unlike the model systems of the prior art, had developed various protective phenomena against viral infections that impeded the use of RNA interference in mammalian cells.

For instance, it was known in the art that the presence of even extremely low levels of viral double-stranded RNA (dsRNA) triggers an interferon response (called “acute-phase response”) and the activation of a dsRNA Responsive Protein Kinase (PKR). PKR phosphorylates and inactivates translation factor EIF2a leading to activation of the 2',5'-oligoadenylate synthetase, finally resulting in RNase L activation. This cascade induces a global non-specific suppression of translation, which in turn triggers apoptosis. For review, see Williams (1997) “Role of the double-stranded RNA-activated protein kinase (PKR) in cell regulation” *Biochem Soc Trans*, 25(2):509-13. Consistent with this mechanism, the use of long dsRNA to silence expression in mammalian cells had been tried and reported at various meetings as being largely unsuccessful as a consequence to the constructs producing a general sequence-independent killing of the mammalian cells.

In several lower eukaryotes, an RNA-dependent polymerase is thought to amplify the introduced long dsRNA, possibly leading to higher levels of siRNAs. See, for example, Smardon et al. (2000) “EGO-1 is related to RNA-directed RNA polymerase and functions in germ-line development and RNA interference in *C. elegans*” *Curr. Biol.* 10:169–178. In *C. elegans*, data support the idea that siRNA pairing with mRNA results

in the extension of the siRNA along the mRNA template to produce a long dsRNA, which could then be processed by Dicer to produce even more siRNAs. However, at the time the present application was filed, it was believed that mammalian cells probably lacked the siRNA amplification mechanisms that confer RNAi potency and longevity in organisms such as worms or plants. The criticality of this amplification mechanism in the prior art model systems was not discerned, adding to the uncertainty as to whether RNAi would be possible in cultured cells of any type, let alone mammalian cells in culture or whole animals.

As described in the present application, the Applicants have discovered that RNAi begins when a nucleolytic enzyme, referred to as DICER, encounters dsRNA and cleaves it into pieces called small-interfering RNAs or siRNAs. This protein belongs to the RNase III nuclease family. When incorporated into a larger, multicomponent complex named RISC (RNA-induced silencing complex) that includes Argonaut, the processed siRNAs form a “guide sequence” that targets the RISC to the desired mRNA sequence and promotes its destruction.

The use of hairpin RNA species to affect gene silencing in mammalian cells, according to the methods of the pending claims, is based on several factors that were first recognized by the Applicants. Among those factors are:

- mammalian cells contain the nucleolytic activity necessary to process hairpin RNA constructs and produce functional siRNA products;
- the use of short hairpin RNA constructs does not produce a general sequence-independent killing of the mammalian cells, e.g., does not trigger an acute-phase response and the activation of a PKR.

Moreover, as the examples of the present application demonstrate, there are certain unexpected benefits to using hairpin RNAs to cause RNA interference in mammalian cells. For instance, the hairpin RNA constructs generally exhibit better reassociation kinetics in cells than equivalent duplex RNA. Perhaps even more significant, the Applicants have demonstrated that transgenic cell lines can be engineered to synthesize hairpin RNAs that exhibit a long-lasting suppression of gene expression that persists throughout cell divisions.

The Fire et al. patent is directed to RNA interference, and teaches a variety of different constructs that are alleged to work in various cell-types, including mammalian cells. However, as set forth above, as of the priority date of the present application, it was generally known in the art that the embodiments suggested from work in invertebrates, such as is the case of the Fire et al. patent, did not work in mammalian cells. Rather than induce sequence-dependent suppression, the long dsRNA constructs most favored in invertebrates induced sequence-independent cell death. In considering whether a reference qualifies as prior, it is respectfully noted that when a prior art reference merely discloses the structure of a compound, evidence showing that attempts to prepare that compound were unsuccessful before the date of invention will be adequate to show inoperability. In re Wiggins, 488 F.2d 538, 179 USPQ 421 (CCPA 1971).

The above allegations notwithstanding, Applicants note that the Fire et al. patent fails to teach or suggest the unexpected advantages arising from the combination of short hairpin RNAs for use in mammalian cells. Applicants contend that the combination of short hairpin RNAs for use in mammalian cells has several unexpected advantages not taught or suggested by the teachings of Fire et al. To reiterate, such hairpin constructs: can be processed by mammalian cells to produce functional siRNA products; do not produce a general sequence-independent killing of the mammalian cells; exhibit favorable reassociation kinetics in cells; and can be used to engineer transgenic cell lines that exhibit a long-lasting suppression of gene expression that persists throughout cell divisions.

The instant claims are directed, at least in part, to attenuating expression of one or more target genes in mammalian cells using a library of hairpin RNA species that do not produce a general sequence-independent killing of the mammalian cells (e.g., a protein kinase RNA-activated (PKR) sequence-independent response). Applicants submit that Fire et al. fails to teach or suggest attenuating gene expression in **mammalian** cells with a hairpin RNA that does not activate PKR sequence-independent response.

The only mention in Fire et al. of a sequence-independent response is reproduced below:

An issue of specificity arises when considering known cellular responses to dsRNA. *Some organisms* have a dsRNA-dependent protein kinase that activates a panic response mechanism<sup>10</sup>. *Conceivably*, the inventive sense+antisense synergy *could reflect* a non-specific potentiation of antisense effects by such a panic mechanism. *This was not found to be the case*: co-injection of dsRNA segments unrelated to unc-22 did not potentiate the ability of unc-22 single strands to mediate inhibition. Also investigated was whether double-stranded structure could potentiate inhibitory activity when placed in *cis* to a single-stranded segment. No such potentiation was seen; unrelated double-stranded sequences located 5' or 3' of a single-stranded unc-22 segment did not stimulate inhibition. Thus potentiation of gene-specific inhibition was observed only when dsRNA sequences exist within the region of homology with the target gene. (column 16, lines 7-22)(*emphasis added*)

This section discusses experiments in *C. elegans* using a 742 nt segment of unc-22 sense and antisense RNAs (see e.g., column 15, lines 31-47). The section cited above merely supports the idea that some organisms have a dsRNA-dependent protein kinase that could reflect a non-specific potentiation but this was not found to be the case in the *C. elegans* system being used. There is no teaching or suggestion that attenuation of gene expression using dsRNAs in mammalian cells could be conducted without activating a PKR sequence-independent response. Indeed, as discussed above, as of the priority date of the present application, it was generally known in the art that the embodiments suggested from work in invertebrates, such as is the case of the Fire et al. patent, did not work in mammalian cells. Rather than induce sequence-dependent suppression, the long dsRNA constructs most favored in invertebrates induced sequence-independent cell death.

Accordingly, the Fire et al. patent fails to teach or suggest attenuation of gene expression in *mammalian cells* using a hairpin RNA that does not activate a PKR sequence-independent response. The standard for anticipating a claim is clearly outlined in MPEP 2131, and this standard is further supported by the Courts. “A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference.” *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1978). “The identical invention must be shown in as complete detail as is contained in the claim.” *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920

(Fed. Cir. 1989). In sum, Fire et al. do not disclose all the limitations of the present claims as amended and thus fail to anticipate the claimed subject matter. Reconsideration and withdrawal of this rejection under 35 U.S.C. § 102(e) are respectfully requested.

The Claims Comply with 35 U.S.C. §112, first paragraph

Claims 83-124 are rejected under 35 U.S.C. §112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Applicants respectfully traverse this rejection.

In particular, the Examiner states that “the specification, while being enabling for a method of attenuating expression of one or more target genes using a dsRNA in mammalian cells *in vitro*, does not reasonably provide enablement for a method of attenuating expression of one or more target genes using a dsRNA or a library of single stranded RNA in mammalian cells *in vivo*.” Applicants respectfully disagree with the rejection, however, in an effort to expedite prosecution of the application, the claims have been amended and the amendments are believed to obviate the rejection. In particular, the claims have been amended to recite “mammalian cells suspended in culture” and therefore are directed to the use of hairpin RNAs for attenuation of gene expression of mammalian cells *in vitro* which is indicated by the Examiner as being enabled by the present specification. Accordingly, Applicants submit that the claims fully comply with the enablement requirement under 35 U.S.C. § 112, first paragraph. Reconsideration and withdrawal of this rejection are respectfully requested.

The Claims Comply with 35 U.S.C. §112, second paragraph

Claim 83-124 are rejected under 35 U.S.C. §112, second paragraph as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In particular, claims 83-124 were rejected for recitation of the term “variegated” because it was alleged that this is a relative term which

renders the claim indefinite. Applicants respectfully disagree with the rejection, however, in an effort to expedite prosecution of the application, claims 83-87 have been amended to remove this term from the claims. Claims 88-124 are dependent on claims 83-87. The amendments are believed to obviate the rejection. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

## CONCLUSION

The Examiner may address any questions raised by this submission to the undersigned at 617-951-7000. Should any additional extensions of time be required, Applicants hereby petition for same and request that the extension fee and any other fee required for timely consideration of this submission be charged to Deposit Account No. **18-1945**, under Order No. CSHL-P03-010 from which the undersigned is authorized to draw.

Respectfully Submitted,

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**Customer No: 28120**  
Docketing Specialist  
Ropes & Gray LLP  
One International Place  
Boston, MA 02110  
Phone: 617-951-7739  
Fax: 617-951-7050

  
Jennifer K. Holmes  
Reg. No. 46,778